



# Draft genome sequences of *Rahnella perminowiae*, *R. aceris*, and *R. aquatilis* isolated from onion bulbs (*Allium cepa* L.) displaying symptoms of bacterial rot

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Onions (*Allium cepa* L.) are among the most widely produced vegetables globally, and their cultivation plays an important role in food security (Schwartz et al. 2007). However, the emergence of opportunistic bacterial plant pathogens, such as *Rahnella* species, which are not fully understood yet, poses a threat to onion production (Asselin et al. 2019; Brady et al. 2022). *Rahnella* species are Gram-negative, facultative anaerobes within the *Yersiniaceae* family, part of the order Enterobacteriales (Adeolu et al. 2016). According to the List of Prokaryotic Names with Standing in Nomenclature (LPSN), the genus consists of 15 described species that are isolated from various environments and are considered validly characterised (Guo et al. 2012; Brady et al. 2014; Lee et al. 2019; Liang et al. 2020).

Recent reports have linked *Rahnella* species to symptomatic onion bulbs in countries such as Norway and the United States (Asselin et al. 2019; Brady et al. 2022). Hence, in a previous unpublished study conducted by our team, we isolated various *Rahnella* species and examined their diversity

and their pathogenicity towards onion bulbs. Our findings indicated that the most prevalent species among those examined were *R. aceris*, *R. aquatilis* and *R. perminowiae*. Symptoms observed of the representative strains on onion bulbs included brown discolouration on the inner fleshy scales, necrosis of the infected onion scales, and some bulbs exhibiting shrunken scales. Other notable plant sources and functions associated with the three *Rahnella* species, for instance, *R. aceris*, were first isolated from the sap of the *Acer pictum* tree (Lee et al. 2020; Asselin et al. 2019). *Rahnella aquatilis* is often recognised for its beneficial role as a plant growth-promoting rhizobacterium (Izard and Trinel. 1979; Berge et al. 1991; Maraki et al. 1994; Guo et al. 2012; Lee et al. 2019; Liang et al. 2020). Moreover, *R. perminowiae* has also recently been acknowledged for plant growth-promoting properties (Zouagui et al. 2024). Given previous reports documenting *Rahnella* species in association with symptomatic onion bulbs, and the limited understanding of the genetic factors that may facilitate these opportunistic interactions, the objective of this study was to generate draft genome sequences of *Rahnella* strains isolated from symptomatic onion bulbs. This work represents an initial step toward identifying potential pathogenicity factors involved in *Rahnella*-onion interactions. The draft genomes presented here are part of an ongoing project investigating *Rahnella* spp.

Two of the five *Rahnella* strains used in this study (20WA0051 and 20WA0052) were isolated from an asymptomatic onion plant in Washington State, USA, in 2020, and two strains (20CA0197 and 20CA0198) were isolated from a symptomatic onion plant in California, USA, in 2020. These four strains were sent to the National Onion Bacterial Strain Collection (NOBSC) at the University of Georgia,

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USA, as part of a multi-state project on onion bacterial diseases (USDA NIFA Speciality Crops Research Initiative Project No. 2019-51181-30013). Isolations of the four *Rahnella* strains from the USA (20WA005, 20WA0052, 20CA0197 and 20CA0198) were done by cutting the symptomatic plant lengthwise from the neck through the bulb using a sterilised knife. A ~5 mm<sup>3</sup> piece of symptomatic tissue was then sampled from the leading edge of the lesion of a fleshy scale within the bulb, macerated in a drop of sterilised water, and the suspension streaked onto nutrient agar medium and dried onion extract agar medium (Zaid et al. 2012). Whereas strain FS4 was isolated from a symptomatic onion bulb grown in the fields of Free State Province, South Africa, which displayed mild symptoms of light brown discoloration of internal fleshy scales, with light maceration and shrinkage of the infected scales. Isolation of this strain of *Rahnella* involved surface sterilisation of the entire onion bulb and excision of a piece of symptomatic fleshy scale tissue. The onion tissue was macerated in sterile 1x PBS and then crushed in a Bioreba extraction bag (Bioreba, Reinach, Switzerland). The resulting suspension was then serially diluted, streaked onto nutrient agar medium, and incubated at 28 °C for 48 h to obtain pure colonies. The DNA from the bacterial strains was extracted using the Zymo *Quick*-DNA Miniprep kit (Zymo Research, California, USA).

The isolated bacterial strains were preliminarily identified as *Rahnella* through the amplification of 16 S rRNA gene sequence using conventional PCR. Amplification was carried out in a 25 µl reaction volume containing 2.5 µl of 10X buffer with MgCl<sub>2</sub>, 2.5 µl of 2 mM dNTPs, 0.5 µl each of 25 nmol universal primers 27 F (AGAGTTTGATCCTGGCTCAG) and 1492R (CGGTTACCTTGTTACGACTT), as previously described by Xu et al. (2023), 0.15 µl of DreamTaq DNA polymerase (5 U/µl; Thermo Scientific, California, USA), 17.85 µl of nuclease-free water (Invitrogen, Massachusetts, USA), and 1 µl of each DNA template. The thermal cycling conditions done on a Bio-Rad T1000 thermocycler (Bio-Rad, California, USA) were: initial denaturation at 96 °C for 3 min; 30 cycles, 96 °C for 30 s, annealing temperature 55 °C for 30 s, and 72 °C for 30 s; followed by a final extension at 72 °C for 5 min. PCR amplicons were visualised on a 1% SeaKem agarose gel (LONZA, Basel, Switzerland) prepared in 1X TAE buffer, run at 100 V for 30 min, and compared against a 1 Kb GeneRuler DNA ladder (Thermo Scientific, Massachusetts, USA). Amplicons were purified using ExoSAP (Exonuclease I and FastAP, Thermo Fisher Scientific, Massachusetts, USA) by incubating at 37 °C for 30 min and then at 85 °C for 15 min. Purified amplicons were PCR sequenced in both directions

using a 12 µl sequencing reaction volume that contained 2.5 µl of 5X sequencing buffer, 0.5 µl of BigDye Terminator (Applied Biosystems, Massachusetts, USA), 0.5 µl of 25 nmol forward primer, 0.5 µl of purified template DNA, and 8 µl of nuclease-free water. Sequencing PCR was performed with initial denaturation at 96 °C for 30 s; followed by 25 cycles at 96 °C for 10 s, 55 °C for 5 s, and 60 °C for 4 min, and a final hold at 12 °C for 5 min. Sequencing amplicons were precipitated by adding 2 µl of 3 M sodium acetate (pH 5.2; Merck, Darmstadt, Germany), 42 µl of 99% ethanol, and 12 µl of nuclease-free water. The sequencing reaction mixture was incubated on ice for 30 min and centrifuged at 10,000 ×g for 30 min. The supernatant was carefully discarded, and the DNA pellet was washed twice with 250 µl of 70% ethanol, followed by centrifugation at 10,000 ×g for 10 min. DNA pellets were air-dried for 2 h at room temperature and submitted to the University of Pretoria Sanger Sequencing Facility for analysis on an ABI 3500xL Genetic Analyzer (Applied Biosystems, Massachusetts, USA). The resulting sequences were analysed and compared to the 16 S rRNA sequences in the NCBI database using the Basic Local Alignment Search Tool (BLAST) database. A sequence percentage identity exceeding 97% was considered indicative of the genus *Rahnella*.

Whole genome raw reads were generated by extracting DNA from pure cultures of the five strains using the Genra Puregene Yeast/Bac Kit (Qiagen, Maryland, USA) following the manufacturer's protocol. DNA concentrations were quantified using a Nanodrop spectrophotometer (Thermo Scientific, Florida, USA). Genomic DNA libraries were prepared for sequencing using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Massachusetts, USA). Sequencing was done using the Illumina MiSeq platform (Inqaba Biotech, Pretoria, South Africa) with a dual indexing step. The five strains were sequenced to create 2 × 150 bp paired end reads and 100× sequencing depth, with a coverage listed in Table 1. The raw reads fastq files were quality-checked using FastQC 0.12.0 (Andrews 2010). The removal of low-quality reads and adaptors was done using Trimmomatic 0.39 (Bolger et al. 2014). The resulting reads were assembled *de novo* using SPAdes 3.15.5 (Bankevich et al. 2012). Genome assembly quality was assessed using Quast 5.0.2 (Gurevich et al. 2013). CheckM 1.1.6 was used to detect any contaminants in the assembly (Parks et al. 2014). The draft genome completeness was determined using BUSCO 5.7.1 (Seppey et al. 2019). Annotation was done using PGAP 5.3 (Tatusova et al. 2016). To ensure robust species identification, the Average Nucleotide Identity (ANI) was calculated using JSpeciesWS (Richter

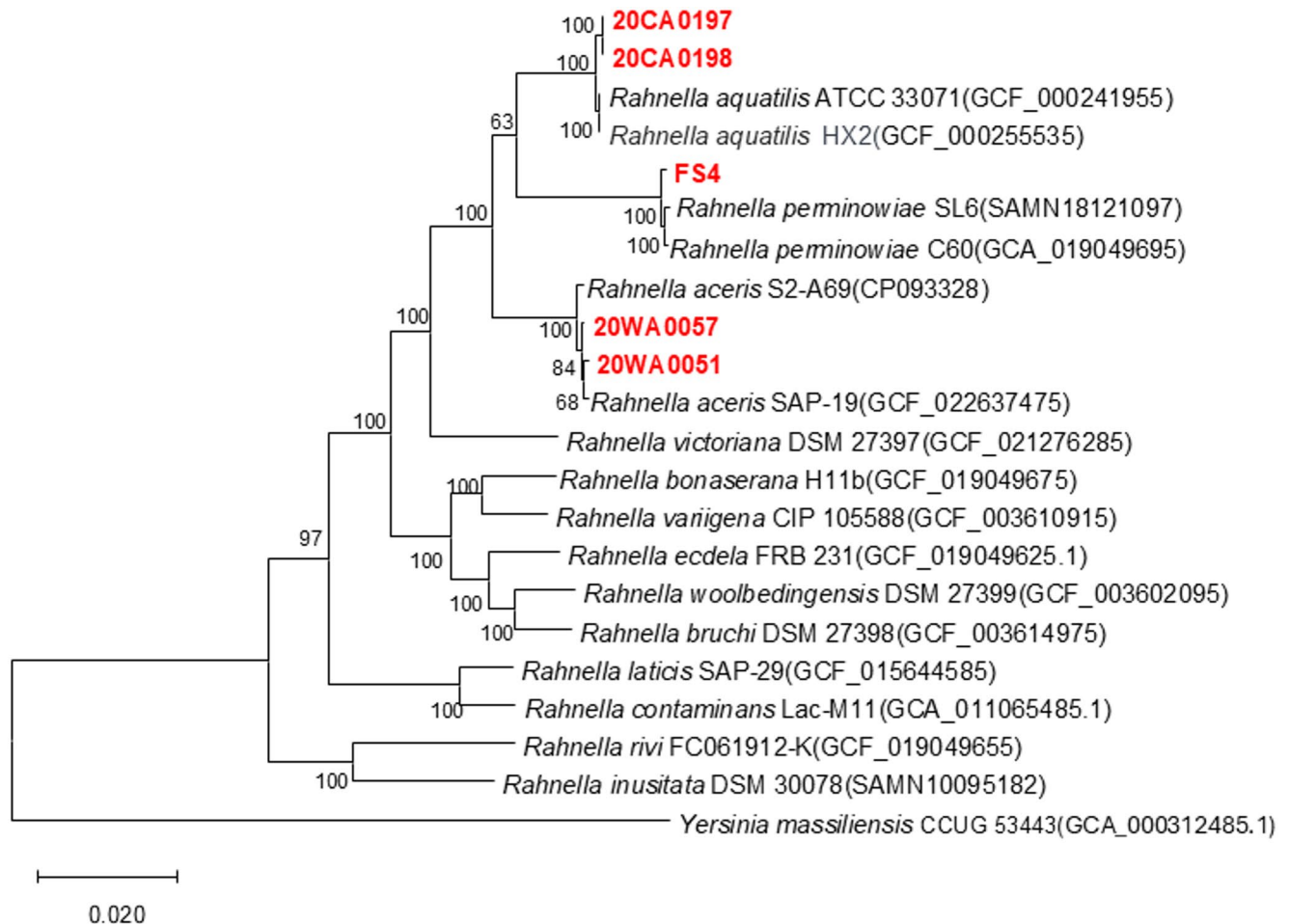
**Table 1** Draft genome assemblies and annotation statistics for the five *Rahnella* strains isolated from onions displaying symptoms of bacterial rot

Genomes features	<i>R. aquatilis</i> 20CA0197	<i>R. aquatilis</i> 20CA0198	<i>R. aceris</i> 20WA0051	<i>R. aceris</i> 20WA0057	<i>R. perminowiae</i> FS4
SRA accession(rawdata)	SRR30861219	SRR30861218	SRR30861220	SRR30861217	SRR30861216
Illumina coverage (×)	33	33	32	102	25
Genome size (bp)	5,538,857	5,539,510	5,576,881	5,840,737	5,721,250
G+C contents %	52.2	52.2	52.1	52.1	51.6
Number of contigs	61	70	110	256	101
N50 (bp)	163,314	163,400	173,614	57,008	222,080
N90 (bp)	60,525	54,699	47,910	15,567	65,959
L50	13	12	8	29	9
L90	31	31	29	102	26
Total genes	5,131	5,141	5,151	5,571	5,326
Total protein-coding genes	4,981	4,990	5,015	5,431	5,152
Annotated rRNAs	3	3	3	3	4
Total number of tRNAs	53	55	53	52	52
Total ncRNAs	8	7	7	8	10
Total Pseudo Genes	86	86	73	77	108
Contaminant (%)	0.89	0.89	1.26	2.06	1.14
BUSCOs (%): Complete	100	100	99.2	98.4	100
Single copy	100	100	99.2	97.6	100
Duplicated	0	0	1	0.8	0
Fragmented	0	0	0.8	0.8	0
Missing	0	0	0	0.8	0

et al. 2016) with default parameters. A core phylogenomic alignment based on 3,356 core genes was generated using Roary 3.11.2 (Page et al. 2015). The resulting phylogenomic alignment was used to test for the best evolutionary model, which was chosen through the Akaike Information Criterion (AIC) using PhyML 3.0 (Lefort et al. 2017). The core maximum phylogenomic tree was done using RAxML 8.0 with a 1000 bootstrap support (Stamatakis 2014), as shown in Fig. 1.

Table 1 summarises the genomic features of the five strains with draft genomes that are 98 to 100% complete. Genomes reported here are *Rahnella aquatilis* strains 20CA0197 and 20CA0198, *R. aceris* strains 20WA0051 and 20WA0057, and *R. perminowiae* strain FS4, had G+C contents ranging from 51.6 to 52.2%, and the number of contigs ranging from 61 for 20CA0197 to 256 for 20WA0057. According to CheckM, the genome contamination levels range from 0.89 to 2.06 for each genome, which is below the recommended 5% threshold. The genome revealed a total of 4,196,195 bp,

96 contigs, 67.5% GC content. Genome assemblies had N50 values > 100,000 bp except for *R. aceris* strain 20WA0057. The draft genomes contained 4,981 to 5,431 protein-coding genes. Three to four rRNAs were detected in each of the five genome sequences. The number of tRNAs ranged from 52 to 55, and each strain had one tmRNA. Our analysis, which is based on the core phylogenetic tree, groups the five *Rahnella* strains alongside three *Rahnella* species: *R. aquatilis*, *R. aceris*, and *R. perminowiae*, as shown in Fig. 1. The strains highlighted in red on the figure are those used in this study. The ANI values for the five strains ranged from 98.89% to 99.39%, exceeding the 95% threshold for species delimitation and confirming the identities as the two California strains as *R. aquatilis*, the two Washington strains as *R. aceris*, and the FS4 strains as *R. perminowiae*. The genomic information of the *Rahnella* representative strains used in this study will provide a foundation for investigating *Rahnella*-onion opportunistic interactions.



**Fig. 1** A maximum-likelihood core gene phylogenetic tree constructed using RAxML (Stamatakis 2014) based on 3,372 core genes shared among 19 *Rahnella* species. The GTR+G evolutionary model was

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**Data availability** The raw sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1035941. The assembled genome sequences are publicly available in the NCBI GenBank database under the accession numbers: JBHUCI000000000 (*R. aquatilis* 20CA0197), JBHUCG000000000 (*R. aquatilis* 20CA0198), JBHUCH000000000 (*R. aceris* 20WA0051), JBHUCJ000000000 (*R. aceris* 20WA0057) and JBHGNNV000000000 (*R. perminowiae* FS4).

## Declarations

**Ethical approval** Not applicable.

**Consent for publication** Publication has been approved by all authors.

**Conflict of interest** Teresa Coutinho is a senior editor of this journal. All other authors declare no conflict of interest.

used to construct the tree, and a bootstrap analysis of 1,000 replicates was conducted. The tree was visualised in MEGA X (Kumar et al. 2018), and a scale bar represents nucleotide substitutions per site

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